Interferon-β is required for interferon-α production in mouse fibroblasts

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The type I interferons – interferon-α (IFN-α) and interferon-β (IFN-β) – are critical for protection against viruses during the acute stage of viral infection [1,2]. Furthermore, type I interferons have been implicated as important mediators in the regulation of lymphocyte development [3], immune responses [4,5] and the maintenance of immunological memory of cytotoxic T cells [6,7]. The different IFN-α subtypes are encoded by 12 genes in the mouse [8] whereas IFN-β is encoded for by only one gene [9]. IFN-α and IFN-β have a high degree of sequence homology and are thought to interact with the same surface receptor on target cells [10,11]. As an approach to analysing the different biological functions of IFN-α and IFN-β, we have generated a mouse strain with an inactivated IFN-β gene. We report here that embryonic fibroblasts from such mice produce neither IFN-β nor IFN-α upon Sendai virus infection, whereas the production of IFN-α by leukocytes from the same strain of mice is intact. IFN-α production in embryonic fibroblasts from IFN-β$^{-/-}$ mice could be rescued by 'priming' the cells using exogenous IFN-β. These results imply a unique role for IFN-β in the induction of type I interferons in peripheral tissues.

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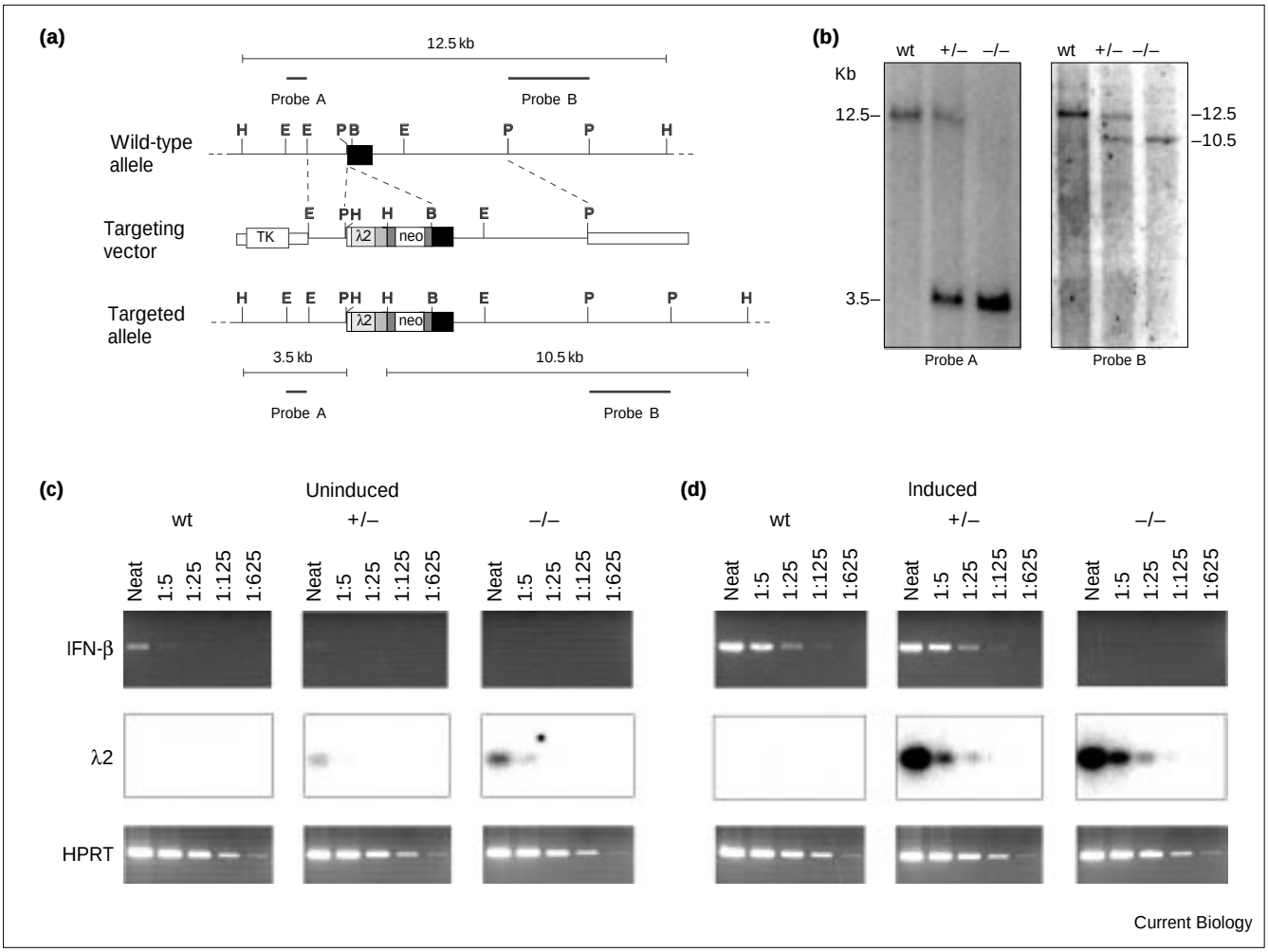
Results and discussion

In order to inactivate the mouse IFN-β locus, a targeting construct was generated as depicted in Figure 1a. A mouse immunoglobulin λ2 chain coding sequence was inserted as a reporter gene immediately downstream of the IFN-β promoter, followed by a neomycin cassette. These heterologous sequences were grafted onto the IFN-β coding sequence, eliminating the region encoding the first 4 amino acids, such that no expression was expected from the modified locus, not even of a truncated IFN-β protein. The construct was transfected into E14 embryonic stem cells and a single clone with homologous integration of the construct in the IFN-β locus was isolated after screening 500 transfectants by Southern blot analysis. Cells of this clone were injected into C57Bl/6 blastocysts. Offspring of chimeric mice were used to breed mice heterozygous and homozygous for a modified IFN-β locus as demonstrated by the Southern blot in Figure 1b.

In order to study the effect of the introduced mutation in the IFN-β locus in a situation in which other cytokines, particularly IFN-γ, do not play a role, we challenged isolated embryonic fibroblasts with Sendai virus *in vitro*. RNA was prepared from challenged fibroblasts established from wild-type littermate controls, IFN-β$^{+/-}$ and IFN-β$^{-/-}$ mice after 6 hours of culture. As expected, no functional IFN-β RNA was detected in fibroblasts from the IFN-β$^{-/-}$ mice using the reverse transcriptase and polymerase chain reaction (RT–PCR) method (Figure 1c,d) or using northern blot analysis (data not shown). Induction of the λ2 reporter gene could be observed, however, in fibroblasts from the IFN-β$^{+/-}$ and IFN-β$^{-/-}$ mice (the low RT–PCR signal from uninduced fibroblasts from IFN-β$^{+/-}$ and IFN-β$^{-/-}$ mice was most probably due to contamination with genomic DNA in the RNA preparations as it was also detected in control samples from which reverse transcriptase was omitted; data not shown). We conclude from these results that we have successfully inactivated the IFN-β locus and that fibroblasts from such IFN-β$^{-/-}$ mice responded to a viral challenge by activating the modified IFN-β locus.

We next analysed the IFN-α and IFN-β production induced by Sendai virus infection of different cell populations from littermate controls, IFN-β$^{+/-}$ and IFN-β$^{-/-}$ mice using immunoassays [12]. As shown in Figure 2a, embryonic fibroblasts from wild-type littermate control mice produced both IFN-α and IFN-β, as did IFN-β$^{+/-}$ mice. In contrast, when fibroblasts from IFN-β$^{-/-}$ mice were challenged with Sendai virus, no IFN-β production was detected, as expected. However, no IFN-α production could be detected in the same cultures. A similar analysis using spleen (Figure 2b) or bone marrow (Figure 2c) cells from littermate controls, IFN-β$^{+/-}$ and IFN-β$^{-/-}$ mice revealed that whereas the IFN-β titer was below the detection level in IFN-β$^{-/-}$ mice, the IFN-α response was intact in these cell populations (note that the culture conditions for leukocytes were different from those for fibroblasts; see Materials and methods).

Figure 1

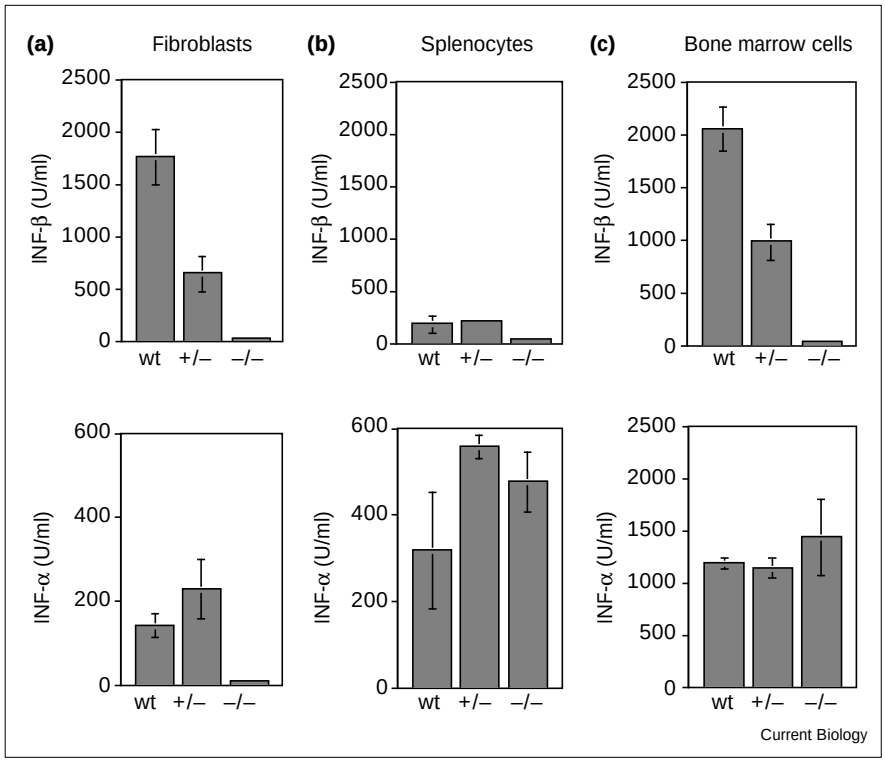


Inactivation of the mouse IFN-β gene by homologous recombination. (a) A schematic representation of the mouse IFN-β locus before (top) and after (bottom) homologous recombination with the targeting vector displayed in the middle. *Bam*HI (B), *Eco*RI (E) and *Pst*I (P) restriction enzyme sites are indicated, as well as the IFN-β gene (black box), the thymidine kinase gene used for negative selection (TK), λ2 cDNA from MOPC315 including an SV40-derived splice donator and acceptor (λ2), a polyadenylation site (light grey box); the neomycin resistance gene (neo), and *loxP* sites not used in the current experiments (dark grey boxes). The diagnostic *Hind*III (H) restriction fragments are indicated on top of the germline configuration and below the targeted locus, as are the probes used for analysis. (b) Southern blots, using the indicated probes, of *Hind*III-digested tail DNA from wild-type littermate controls (wt), heterozygous (+/–) and homozygous (–/–) IFN-β-targeted animals. (c,d) Analysis, using the reverse transcriptase and polymerase chain reaction (RT–PCR) method, of hypoxanthine phosphoribosyltransferase (HPRT), IFN-β and λ2 RNA from embryonic fibroblasts of wild-type, IFN-$\beta^{+/-}$ and IFN-$\beta^{-/-}$ mice before (c) and after (d) infection with Sendai virus.

The finding that embryonic fibroblasts from IFN-$\beta^{-/-}$ mice were unable to produce IFN-α was puzzling as the same cells activated the expression of the λ2 reporter gene after viral challenge (Figure 1d), and thus the signalling mechanism for the viral infection response was intact. In addition, the production of IFN-α by spleen and bone marrow cultures indicated that the IFN-α locus was not affected by the introduced recombination event. The possibility remained that, in embryonic fibroblasts, IFN-β may be needed for the 'priming' of IFN-α production [8,13,14]. To explore this possibility, embryonic fibroblasts from all three types of mice were primed with recombinant IFN-β for 2 hours and subsequently challenged with Sendai virus. As shown in Figure 3, priming with IFN-β recovered the IFN-α response in embryonic fibroblasts from IFN-$\beta^{-/-}$ mice, such that IFN-α was produced at the same levels as in fibroblasts derived from wild-type littermate controls or IFN-$\beta^{+/-}$ mice. Thus, IFN-β may be pivotal in triggering the production of type I interferons in non-hematopoetic tissues.

Figure 2

Quantitation of IFN-α and IFN-β, using dissociation-enhanced, lanthanide fluoroimmunoassays, in the supernatants of Sendai virus infected cell populations from wild-type, IFN-$\beta^{+/-}$ and IFN-$\beta^{-/-}$ mice. The cells were infected with Sendai virus for 2 h and the supernatants harvested after an additional 22 h. **(a)** Embryonic fibroblasts (*n* = 2); **(b)** splenocytes (*n* = 3); **(c)** bone marrow cells (*n* = 3).



Materials and methods

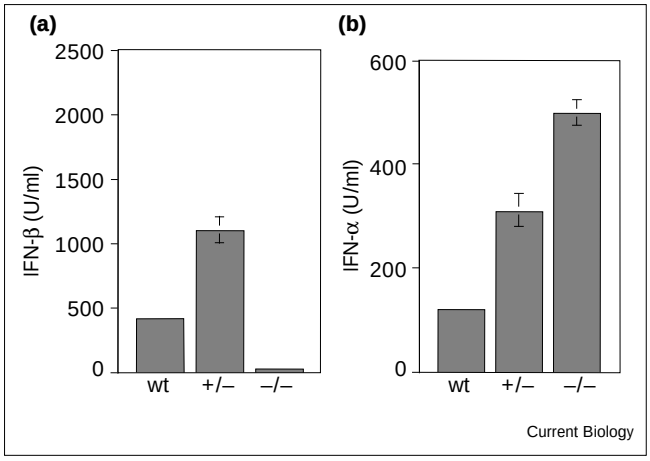
Mice and homologous recombination

The targeting vector was constructed using conventional molecular biology techniques. Homology cassettes of the murine IFN-β locus were subcloned from a cosmid derived from a J129 genomic library kindly provided by J. Bode (GBF, Braunschweig). For the 5′ homology region, the 1.2 kb *Eco*RI–*Pst*I promoter fragment was used. For the 3′ homology region, the 4.5 kb *Bam*HI–*Pst*I fragment that contained most of the IFN-β structural gene was used. This strategy deleted the 73 bp *Pst*I–*Bam*HI fragment including the IFN-β translational start codon. The fragment was replaced by a cDNA corresponding to the λ2 chain of MOPC315 and a standard neomycin cassette which was flanked by *loxP* recombination sites. Electroporation into E14 embryonic stem cells, G418 selection, isolation of resistant colonies, blastocyst injections and mouse breeding were carried out according to standard procedures.

Southern blotting and RT–PCR

Genomic DNA from either embryonic stem cells or tail biopsies was prepared and analysed by Southern blotting according to standard procedures. For RT–PCR analysis, semiconfluent embryonic fibroblasts (in 90 mm petri dishes) were cultured in serum-free Iscoves modified Dulbecco's medium (IMDM) supplemented with 64 hemagglutination units/ml (HA units/ml) Sendai virus for 5 h, after which they were washed in phosphate buffered saline (PBS) and RNA prepared using Trizol™ (Gibco). One half of the recovered RNA was reverse transcribed in a volume of 20 µl using Superscript (Gibco) and the cDNA was used as template at various dilutions in RT–PCR assays. As a negative control, the second half of the RNA was treated identically, except that the reverse transcriptase was left out (data not shown). The primers used were IFN-β forward (sequence deleted in the targeted locus; 5′-TGCATCTTCTCC-GTCATCTC-3′), IFN-β reverse (5′-TAGCAGCCGACACCAGCCTG-3′), HPRT forward (5′-GCTGGTGAAAAGGACCTCTCG-3′), HPRT reverse (5′-GATGGCCACAGGACTAGAACACC-3′), Vλ2 forward (5′-TCT-GCTCAGGAGCCAGTTCC-3′) and Cλ2 reverse (5′-ACA-CGGTGAGAGTGGGAGTG-3′). To assure the specificity of the λ2 amplification, the product was transferred to Genescreen plus filters and hybridised with a Vλ-specific probe (data not shown).

Figure 3



Priming induces IFN-α production in IFN-$\beta^{-/-}$ fibroblasts. Quantitation of **(a)** IFN-β and **(b)** IFN-α, using dissociation-enhanced, lanthanide fluoroimmunoassays, in supernatants from Sendai virus infected embryonic fibroblasts derived from wild-type, IFN-$\beta^{+/-}$ and IFN-$\beta^{-/-}$ mice after priming with 100 U/ml recombinant IFN-β for 2 h before virus infection (*n* = 3).

Tissue culture and virus induction

Embryonic fibroblasts were prepared from day 14 embryos by teasing the tissue with scissors and two rounds of trypsinisation. The single cell suspension was plated in tissue culture bottles and non-adherent cells washed away after 24 h. The fibroblasts were expanded and either frozen or used for experiments after the first passage. Semi-confluent fibroblasts were induced in serum-free IMDM using 32 HA units/ml Sendai virus for 2 h, after which the cells were washed with PBS and recultured in IMDM supplemented with 10% fetal calf serum for 22 h before the supernatants were harvested and analysed with regard to interferon titer. For priming, the fibroblasts were incubated with 100 U/ml recombinant mouse IFN-β (BioSource International) for 2 h, whereafter the cells were washed and induced with Sendai virus as described above. Splenocytes and bone marrow cells were cultured in flat-bottomed 96 well plates in serum-free IMDM with 32 HA units/ml Sendai virus for 22 h, after which the supernatants were harvested and analysed for interferon content.

Immunoassay of mouse IFN-α and IFN-β

The two dissociation-enhanced, lanthanide fluoroimmunoassays (DELFIAs) used have been described previously [12]. In brief, microtitre wells were coated with a sheep anti-mouse IFN-α/β (a kind gift of M. Tovey, Paris) and developed using rat monoclonal antibodies to IFN-α (clone 4E-A1) or IFN-β (clone 7F-D3) labelled with Europium lanthanide chelate (Wallac Oy). Fluorescence, after the addition of enhancement solution, was measured in a 1234 DELFIA Research Fluorometer (Wallac). The assays were calibrated against the murine NIH IFN-α standard Ga02-901-511 and the IFN-β standard Gb02-902-511.

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References

1. Gresser I: Role of interferons in resistance to viral infection ***in vivo***. In *Interferons and the Immune System*. Edited by Vilcek J, De Maeyer E. Amsterdam: Elsiever Press; 1984, 222-247.
2. Müller U, Steinhoff U, Reis L, Hemmi S, Pavlovic J, Zinkernagel RM, Auget M: Functional role of type I and type II interferons in antiviral defence. *Science* 1994, 264:1918-1921.
3. Wang J, Lin Q, Langston H, Cooper MD: Resident bone marrow macrophages produce type I interferons that can selectively inhibit interleukin-7-driven growth of B lineage cells. *Immunity* 1995, 3:475-484.
4. Bellardelli F: Role of interferons and other cytokines in the regulation of the immune response. *APMIS* 1995, 103:161-179.
5. Bellardelli F, Gresser I: The neglected role of type I interferon in the T-cell response: implications for its clinical use. *Immunol Today* 1996, 17:369-371.
6. Tough DF, Borrow P, Sprent J: Induction of bystander T cell proliferation by viruses and type I interferon ***in vivo***. *Science* 1996, 272:1947-1950.
7. Gray D: Viral immunity: interferons jog old T-cell memories. *Curr Biol* 1996, 6:1254-1255.
8. De Maeyer E, De Maeyer-Guignard J: *Interferons and Other Regulatory Cytokines.* New York: John Wiley and Sons; 1988.
9. Weissman C, Weber H: The interferon genes. *Prog Nucleic Acid Res Mol Biol* 1986, 33:251-300.
10. Cleary CM, Donnelly RJ, Soh J, Mariano TM, Pestka S: Knockout and reconstitution of a functional human type I interferon receptor complex. *J Biol Chem* 1994, 269:18747-18749.
11. Uzé G, Lutfalla G, Mogensen KE: **α** and **β** interferons and their receptors and their friends and relations. *J Interferon Cytokine Res* 1995, 15:3-26.
12. Eloranta M-L, Sandberg K, Alm GV: The interferon-**α**/**β** responses of mice to herpes simplex virus studied at the blood and tissue level ***in vitro*** and ***in vivo***. *Scand J Immunol* 1996, 43:355-360.
13. Stewart WE: Varied biologic effects of interferon. In *Interferon*. Edited by Gresser I. London: Academic Press; 1979:29-51.
14. Roszтoczy I, Pitha P: Priming does not change promoter sequence requirements for IFN induction or correlate with the expression of IFN regulatory factor-1. *J Immunol* 1993, 151:1303-1311.